SCANNING ELECTRON MICROSCOPIC DEMONSTRATION OF BACTERIA ON TARSI OF *BLATTELLA GERMANICA*

PASKO GAZIVODA AND DURLAND FISH

Department of Biological Sciences, Fordham University, Bronx, New York 10458

Abstract.—Using scanning electron microscopy (SEM), we have demonstrated the presence of bacteria on the outer surface of the tarsi of adult male and female German cockroaches (*Blattella germanica*), collected from natural populations. Our results indicate that the microorganisms are relatively tightly attached, supporting the hypothesis that mechanical transmission is important in introducing bacteria into human habitation, and may play a role in infections of humans and domesticated animals.

Various investigators have shown that *Blattella germanica* (Linn.) as well as other species of domesticated cockroaches can transmit infectious diseases. Two mechanical routes by which cockroaches can transport bacteria from waste disposal sites or unsanitary conditions into kitchens where food is prepared have been proposed: 1) adhesion to the exoskeleton, especially on leg surfaces; 2) through the alimentary canal where the organisms can remain viable for several days or weeks and are later expelled (Cornwell, 1968).

Several investigators have indicated that *B. germanica* serves as primary and developmental vectors for viruses (e.g., poliomyelitis), fungi (*Aspergillus niger*), protozoans (*Entamoeba histolytica*), and helminths (*Raillietiella hemidactyli*, "tongue worms") (Roth and Willis, 1957, 1960; Guthrie and Tindall, 1968; Lavoipierre and Lavoipierre, 1966; Harwood and James, 1979).

The purpose of this study was to determine, using SEM, if bacteria could attach to and colonize the outer surface of the cockroach exoskeleton. Bacterial attachment to and concomitant colonization of the exoskeleton represents a relatively simple route for the mechanical transmission of pathogenic bacteria.

MATERIALS AND METHODS

Adult *B. germanica* were trapped in large glass jars in an apartment house in New York City. Strips of masking tape were placed on the outer surface of the jar to facilitate cockroach entrance and slices of fresh banana were used as bait. Tarsi of adult male and female cockroaches were prepared for scanning electron microscopy by fixation with 5% glutaraldehyde in 0.067 M phosphate buffer (pH 7.4) for 24 hours at 4°C, and post-fixed with 1% osmium tetroxide in phosphate buffer (pH 7.4) for 1 hr at 4°C. They were then dehydrated for 15 min at room temperature with increasing concentrations of ethanol. The specimens were kept in absolute alcohol and not allowed to dry. Specimens were dried in a Polaron Critical Point Drying Apparatus, Model E 3000, then mounted on brass stubs with double-sided scotch tape, and coated with a 240 Å layer of gold using an ISI-Sputter Coater, Model P. SI (10 mA, 1.2 kV, 4 min; k = 5) (Nowell and Pawley, 1980). Photomicrographs



Figs. 1–4. Attachment of bacteria to the tarsi of *Blattella germanica*. 1. Female, arrow indicates bacilli and associated material ($\times 100$). 2. Enlarged view of area indicated by arrow in Fig. 1. ($\times 700$). 3. Bacterial cocci on male ($\times 6,000$). 4. Bacilli on male ($\times 3,500$).

were taken with Stereoscan Scanning Electron Microscope (JSM-U3) operating at 25 kV.

RESULTS

Twenty-five hindleg tarsi from 10 male and 10 female adult *B. germanica* were studied, and bacteria were observed on all specimens. Figures 1 and 2 show that the bacteria were attached to the outer surface of the tarsi and their configuration indicated

colony formation thus suggesting that cockroaches could act as vectors for certain multiplying microorganisms. The micrographs, show that the bacteria are cocci, and rod shaped, and are surrounded by what appears to be a viscous material (Figs. 3 and 4). The presence of this type of substance can facilitate bacterial attachment to a variety of natural environmental surfaces (Bruce et al., 1983). Viscous material constitutes the covering layer or envelope of some bacteria and is known as capsule (integral fibrous glycocalyx) or slime (flexible peripheral glycocalyx) (Bruce et al., 1983; Gibbons, 1977).

DISCUSSION

About 40 pathogenic and 45 non-pathogenic species of bacteria have been isolated from naturally contaminated cockroaches (Roth and Willis, 1957). Many of the pathogens were isolated from B. germanica. An important feature in mechanical bacterial transmission is the fact that some microorganisms can remain viable on inert surfaces for long perods. Salmonella oranienburg (which has been found naturally in cockroaches) can survive at room temperature for up to 34 days on an inert surface such as glass, and will remain viable for at least 62 and 88 days when attached to corn flakes, and dry biscuits, respectively (Cornwell, 1968). Roth and Willis (1957, 1960) indicate that the evidence implicating cockroaches in the transmission of bacterial disease agents is largely circumstantial. Scanning the tarsi with SEM provides direct evidence that bacteria can attach and colonize the cockroach exoskeleton. This attachment permits mechanical transmission which may be of significance in certain bacterial infections affecting humans and domesticated animals. Although surface attachment and colonization is widespread in nature, little is known about the mechanisms involved in the adhesion of microorganisms to surfaces (Gibbons, 1977; Beachey, 1981; Galask et al., 1984).

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